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(54) Title: A LACTOFERRIN CONTAINING THERAPEUTIC AGENT FOR RHEUMATISM AND DERMATOLOGICAL AND COSMETIC COMPOSITIONS CONTAINING SUCH AGENT

(57) Abstract

The present invention concerns a therapeutic agent for rheumatism which contains lactoferrin as an active ingredient. Such a therapeutic agent shows excellent inhibitory effect against collagenase activity and is useful for treatment for rheumatism.

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A LACTOFERRIN CONTAINING THERAPEUTIC AGENT FOR RHEUMATISM AND DERMATOLOGICAL AND COSMETIC COMPOSITIONS CONTAINING SUCH AGENT .

This invention relates to a therapeutic agent for rheumatism, which contains lactoferrin as an active ingredient.

Lactoferrin, a protein existing in milk or tears of human being, bovine, etc., is known to have pharmacological effects such as antibacterial effect and proliferating effect of lymphocytes (Japanese Unexamined Patent Publication 48534/1990 etc.).

However, it is desired to study potential effects of lactoferrin, a natural product, and expand the applications thereof.

Thereupon, we studied to find new pharmacological effects of lactoferrin and found that lactoferrin was useful for treatment of rheumatism.

This invention relates to a therapeutic agent for rheumatism, which contains lactoferrin as an active ingredient.

It is expected that lactoferrin, a natural protein, has a possibility to be applied for treatment of various diseases. However, as the pharmacological effects of lactoferrin, only antibacterial effect and proliferating effect of lymphocytes etc. have been reported. Therefore, we studied to find new pharmacological effects of lactoferrin and found that lactoferrin was useful for treatment of rheumatism.

An inhibitory effect of a medical substance against collagenase activity can be used as an index to examine the utility thereof for antirheumatic agent. The relationship of inhibitory effect against collagenase activity and rheumatism has been reported (W. H. Johnson et. al., J. Enzyme Inhibition, 2, 1 (1987) ; Woolley D. E. et. al., Arthritis Rheum., 20, 1231 (1977)). Therefore, we examined the inhibitory effect of lactoferrin against collagenase activity.

As the result, we found that lactoferrin significantly inhibited the degradation of collagen caused by collagenase and showed excellent inhibitory effect against collagenase activity. The detailed experimental data are shown in the article of pharmacological test.

Lactoferrin is usually administered directly to knee joint etc. by injection, but it may be administered orally. The dosage is adjusted depending on symptom, dosage form etc. In case of injection, the usual concentration of lactoferrin is 0.1% - 10%, preferably 0.5% - 5%, and suitable volume of the solution is injected to a rheumatoid joint. In case of oral form, the usual daily dosage is 1 to 5000 mg, preferably 50 - 1000 mg in one or a few divided doses.

The preparations of lactoferrin can be prepared by usual methods. Injection can be prepared by dissolving lactoferrin in distilled water for injection and, if necessary, usual excipients such as isotonic agent, pH adjusting agent and viscosity agent can be added. Tablet can be prepared by using usual excipients such as binding agent and lubricant.

Examples of formulations are shown below.

Examples of Formulations

1) Injection

formulation 1

lactoferrin	1g
sodium chloride	0.9g
distilled water for injection	q.s.
total	100ml

formulation 2

lactoferrin	2g
sodium chloride	0.9g
distilled water for injection	q.s.
total	100ml

formulation 3

lactoferrin	5g
sodium chloride	0.7g
distilled water for injection	q.s.
total	100ml

formulation 4

lactoferrin	0.5g
sodium chloride	0.9g
distilled water for injection	q.s.
total	100ml

formulation 5

lactoferrin	10g
sodium chloride	0.5g
distilled water for injection	q.s.
total	100ml

formulation 6

lactoferrin	0.1g
sodium chloride	0.9g
methyl cellulose	0.5g
distilled water for injection	q.s.
total	100ml

2) Tablet

formulation 7

lactoferrin	100mg
lactose	50mg
crystalline cellulose	30mg
hydroxypropylcellulose	5mg
magnesium stearate	5mg
total	190mg

PHARMACOLOGICAL TEST

We examined the inhibitory effect of lactoferrin against collagenase activity according to the method of Nagai et. al. (Japanese Journal of Inflammation, 4, 123 (1984)).

Inhibitory effect against collagenase derived from microorgan (Experimental Method)

Collagen labeled with fluorescein isothiocyanate and collagenase derived from microorgan (Clostridium histolyticum) were dissolved in Tris-HCl buffer (0.05M, pH7.5), which contains sodium chloride (0.2M), calcium chloride (0.005M) and sodium azide (0.02%), in a concentration of 0.025% and 12.5 unit/ml respectively. To 0.4ml of this solution, lactoferrin was added and the mixture was incubated for 1hr at 35°C in a brown test tube. 10µl of water-ethanol solution (1:1 V/V) dissolving o-phenanthroline (80mM) was added to stop the reaction and the mixture was incubated for 1hr at 35°C. 400µl of a mixture of Tris-HCl buffer (0.05M, pH9.5), which contains sodium chloride (0.2M), and ethanol (3:7 V/V) was added and the mixture was stirred and centrifuged. Degradated collagen was assayed by measuring the fluorescence intensity of the supernatant (excitation wavelength : 495 nm, emission wavelength : 520nm).

In the control, it was treated by the same manner as the above except the addition of lactoferrin.

(Result)

Inhibitory percentage against the degradation of collagen by the addition of lactoferrin was shown in Table 1.

Table 1

		fluorescence intensity	inhibition(%)
control		42.6 ± 0.7	
lactoferrin	0.3125mg/ml	38.0 ± 0.8	10.6
	0.625	33.7 ± 0.5	20.9
	1.25	24.6 ± 0.9	42.3
	2.5	15.7 ± 1.1	63.1
	5.0	7.5 ± 0.9	82.4
	10.0	2.5 ± 0.4	94.1

Inhibitory effect against collagenase derived from tissue (Experimental Method)

The inhibitory effect against tissue collagenase, which was purified from cornea of rabbit according to the method reported by Burns et al. (Invest. Ophthalmol. Vis. Sci., 30, 1569 (1989)), was examined by the similar method as for collagenase derived from microorgan. In this experiment, the amount of collagenase to be added was 2.5mg protein/ml.

(Result)

The experimental results were shown in Table 2.

Table 2

		fluorescence intensity	inhibition(%)
control		34.7 ± 1.7	
lactoferrin	5.0mg/ml	28.0 ± 2.8	19.3
	10.0	14.4 ± 1.7	58.5

As shown in Tables 1 and 2, we found that lactoferrin inhibited the degradation of collagen in a

dose-dependent manner and showed an inhibitory effect against collagenase activity in the both experiments using collagenase derived from microorgan or tissue.

As shown in the article of pharmacological test, lactoferrin shows excellent inhibitory effect against collagenase activity and is useful for treatment for rheumatism.

As it was shown that lactoferrin is able to inhibit bacterial and tissue collagenase activity in a dose dependent manner, it is evident that this activity is of interest to cosmetic application.

Skin ageing is a complex process, but it is well known that enzymatic hydrolysis of tissue specific macromolecules, such as collagen and elastine, can contribute to premature scenescence. Natural, inoffensive and efficacious collagenase inhibitors are therefore of interest. Lactoferrin can therefore be used as an agent for the prevention of premature ageing due to its contribution to the preservation of the integrity of the skin.

The concentrations at which lactoferrin is active against these collagenases are of reasonable range for dermatology and cosmetic applications.

Cosmetic applications include oral hygiene also, where lactoferrin may be of use in the inhibition of bacterial collagenases : prevention of bacteriolitic destruction of the gums.

Therefore, in dermatological and cosmetic compositions, lactoferrin is used as inhibitor of bacterial and tissues collagenases, in concentrations between 0.005 % and 5 % (preferably between 0.05 % and 0.5 %).

Lactoferrin may also be used for dermatological and cosmetic compositions in the form of creams, lotions, milks, tooth paste, mouthwash rinses, gels, for application in skin care, oral hygiene and prevention of tissue degradation.

CLAIMS

1/ - A therapeutic agent for rheumatism, which contains lactoferrin as an active ingredient.

2/ - Dermatological and cosmetic compositions containing lactoferrin as inhibitor of bacterial and tissue collagenases, in concentrations between 0.005 % and 5 %, preferably between 0.05 % and 0.5 %.

3/ - Dermatological and cosmetic compositions according to claim 2, characterised in that they are : creams, lotions, milks, tooth paste, mouthwash rinses, gels, for application in skin care, oral hygiene, prevention of tissue degradation.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/00015

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A61K37/64

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO,A,8 604 217 (GAURI) 31 July 1986 see page 1, line 5 - line 9 see page 6, line 24 - line 34 see page 7, line 5 - page 9, line 28 ---	1-3
X	DATABASE WPIL Section Ch, Week 8927, Derwent Publications Ltd., London, GB; Class D21, AN 89-197157 & JP,A,1 135 726 (YG NONOGAWA SHOJI) 29 May 1989 see abstract --- -/--	2,3

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 APRIL 1993	21.04.93
International Searching Authority	**Signature of Authorized Officer**
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	DATABASE WPIL Section Ch, Week 8749, Derwent Publications Ltd., London, GB; Class D21, AN 87-343748 & FR,A,2 596 986 (SEDERMA SA) 16 October 1987 see abstract	2,3
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A	THE SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION vol. 46, no. 7, 1986, OSLO,NORWAY pages 695 - 704 BAYNES ET AL 'THE NON-IMMUNE INFLAMMATORY RESPONE:SERIAL CHANGES IN PLASMA IRON,IRON-BINDING CAPACITY,LACTOFERRIN,FERRITIN AND C-REACTIVE PROTEIN' see the whole document	
A	CLINICAL AND EXPERIMENTAL RHEUMATOLOGY vol. 8, 1990, PISA,ITALY pages 159 - 162 JENSEN ET AL 'RELEASE OF ELASTOLYTIC ACTIVITY FROM HUMAN MONOCYTES AND GRANULOCYTES IN VITRO BY IMMUNE COMPLEX STIMULATION' see the whole document	
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III. DOCUMENTS CONSIDERED T BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	JOURNAL OF ENZYME INHIBITION vol. 2, no. 1, 1987, LONDON,GB pages 1 - 22 JOHNSON ET AL 'COLLAGENASE INHIBITORS:THEIR DESIGN AND POTENTIAL THERAPEUTIC USE' cited in the application see the whole document -----	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9300015
SA 69237

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
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